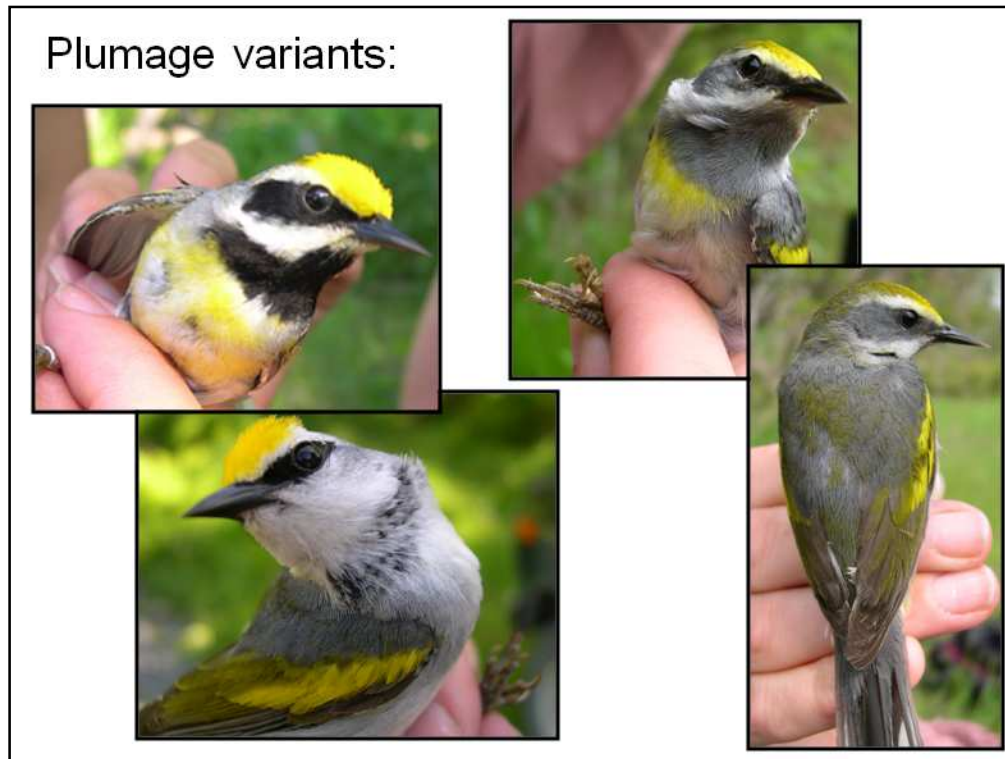


On the top of this slide we have the classic Golden-winged and Blue-winged Warbler phenotypes. You can see that they have strikingly dissimilar plumage, although as my analyses indicated, they are genetically very similar.

When these species hybridize they tend to produce one of two phenotypes – Lawrence's and Brewster's Warblers – seen here on the lower section of this slide.

When individuals displaying these plumage characters were first encountered in the 1870's it was assumed that they were distinct species, even though all the plumage characters in the two new forms were seen in either Blue-winged or Golden-winged Warblers.



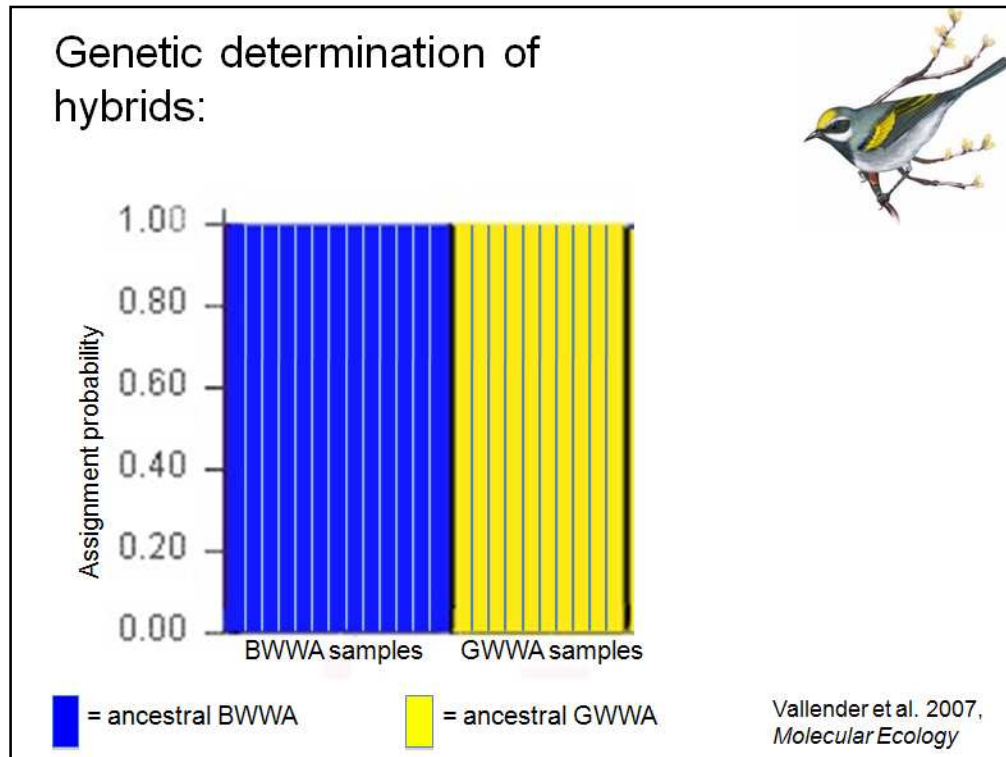
And when you spend a lot of time in the field catching these birds you realized that there are a fair number of hybrids with variants of the classic hybrid phenotypes such as those seen here.

So while they may not look like BRWA or LAWA we can easily identify these individuals as containing BWWA-specific material and they could be classified as hybrids..



But what about individuals that look like GWWA but contain BWWA genetic material? These are known as cryptic hybrids. We are currently unaware of the effect that having BWWA genes has on these individuals – does it effect mate or territory choice? And what about their long term survival? Do they have lowered RS or do they benefit from hybrid vigour.

Some of the preliminary genetic work we've done suggests that even in areas where there has been limited hybridization up to 30% of phenotypic GWWA – so birds that look like GWWA – have hybrid genomes, or contain BWWA genetic material.



Without going into too many details about how we use genetics to make these determinations, I would like to explain the general approach. In this figure each bar represents one individual. Here I show 13 BWWA and 10 GWWA. On the X axis I show the probability that an individual will belong to a specific group, the groups being shown in either blue or yellow. These groups represent the ancestral state. So each one of these BWWA have a 100% chance probability of being assigned to the BWWA group – there is no probability that they would be assigned to the GWWA group.

Using a panel of genetic marker

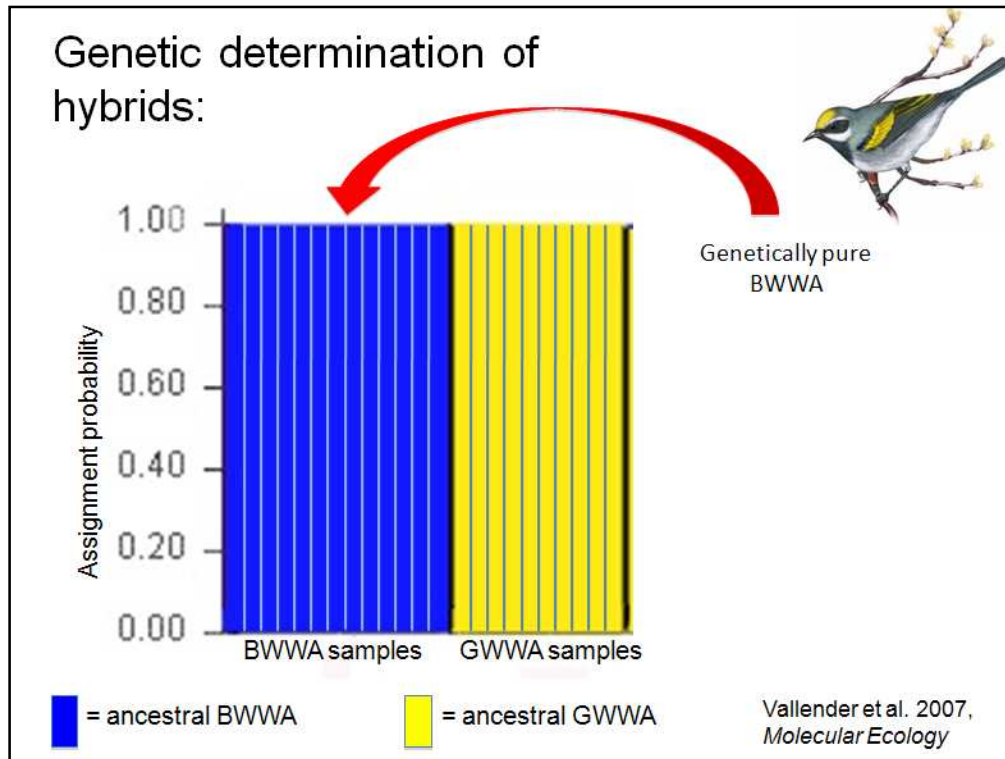
We use STRUCTURE to examine AFLP differences among the allopatric and actively hybridizing populations.

We first tested whether structure could group individuals from the two allopatric populations into clusters that corresponded with sampling sites. Structure did assign individuals from the allopatric sites to different populations with high confidence. POINT OUT BLUE AND YELLOW. You cannot see the individual samples in this figure as they are all the same colour.

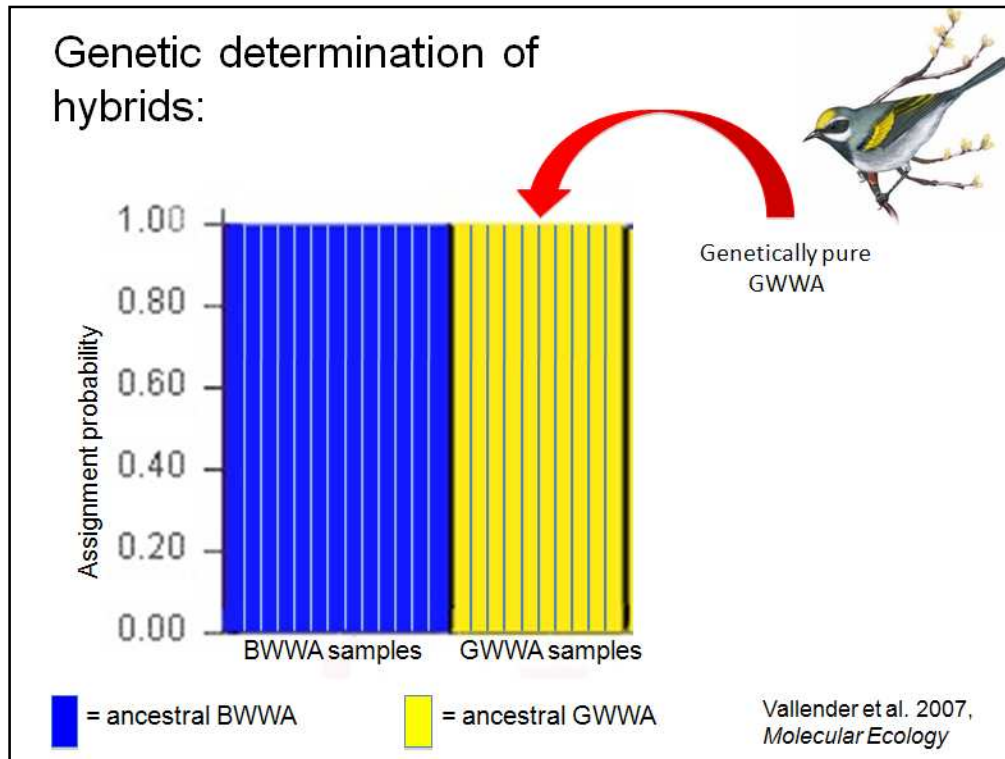
We then used these two groups as our reference groups and used them to assign the ancestry of samples from Ontario using a Bayesian clustering approach.

As you can see from the last section of this figure, there was a mix of individuals who could be assigned to the ancestral BW and GW populations, and that there were many individuals that could not be assigned to either of the two, but rather are a mix. Again, each narrow bar represents one individual.

So what does this mean? Well it shows that there is evidence of cryptic hybridization in the

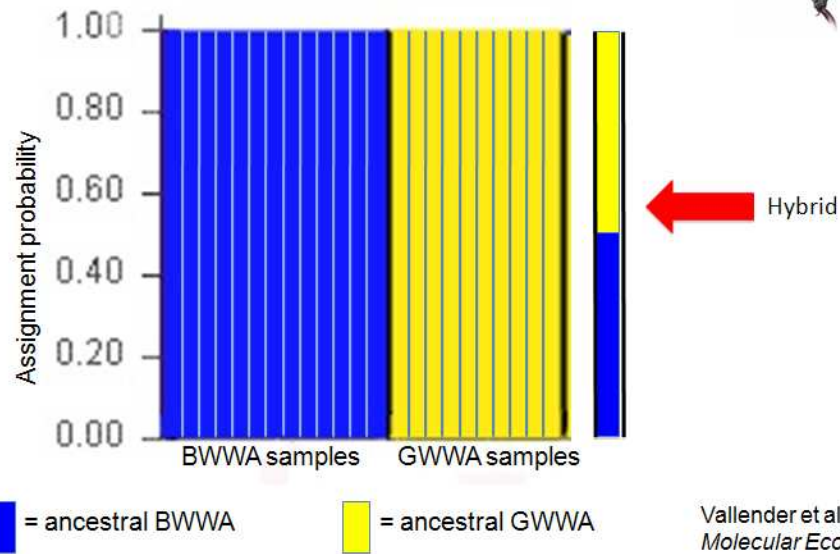


So we classify these individuals as being genetically pure BWWA.

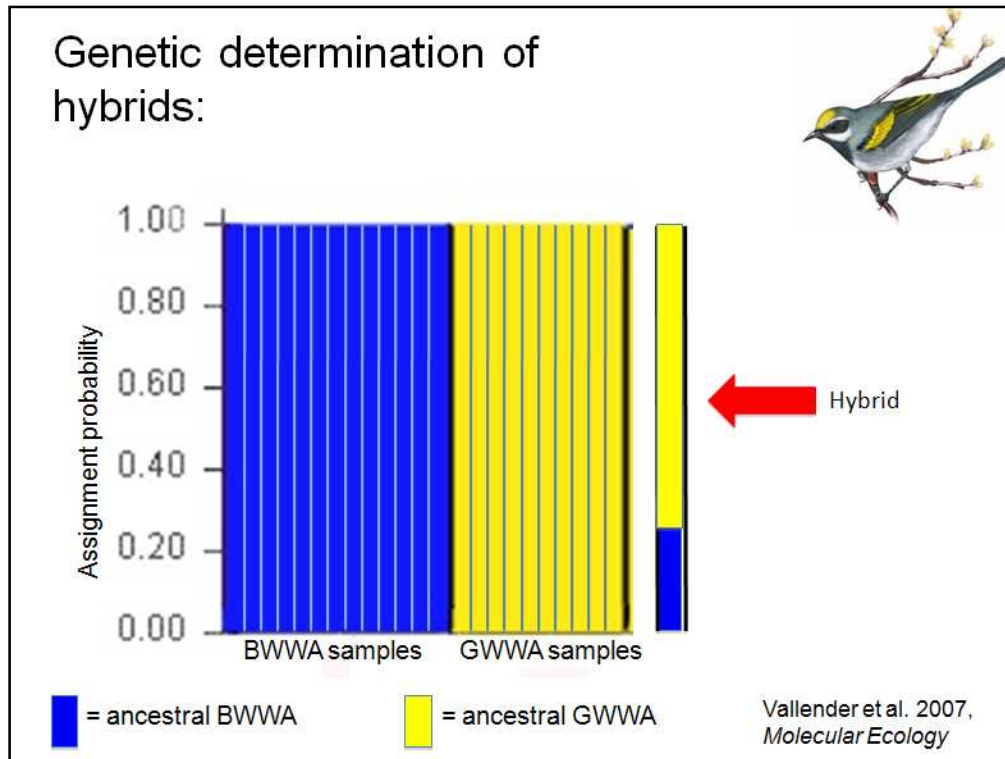


By the same token we would classify all of the GWWA samples as being assigned to the ancestral GWWA state with 100% certainty

Genetic determination of hybrids:

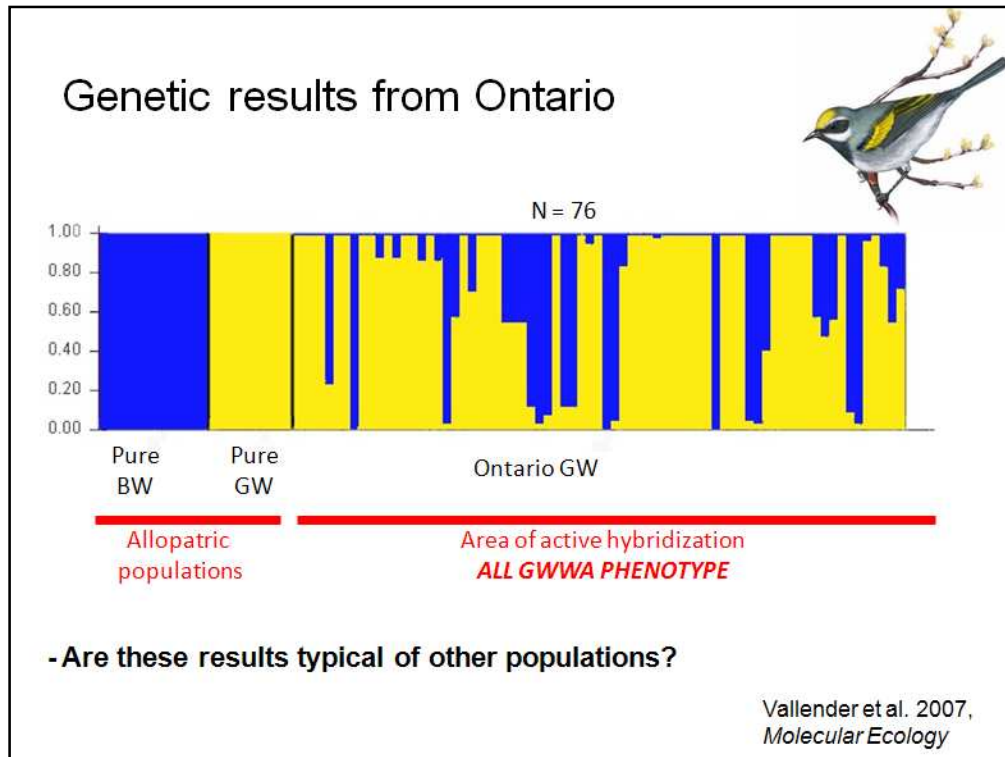


If an individual cannot be assigned to either one of the parental states, but rather shows a mix of the two we know that this individual is a hybrid based on the markers we've screened.



This is the case even when it appears to have received some of its genome from one of the two species - this is still a hybrid.

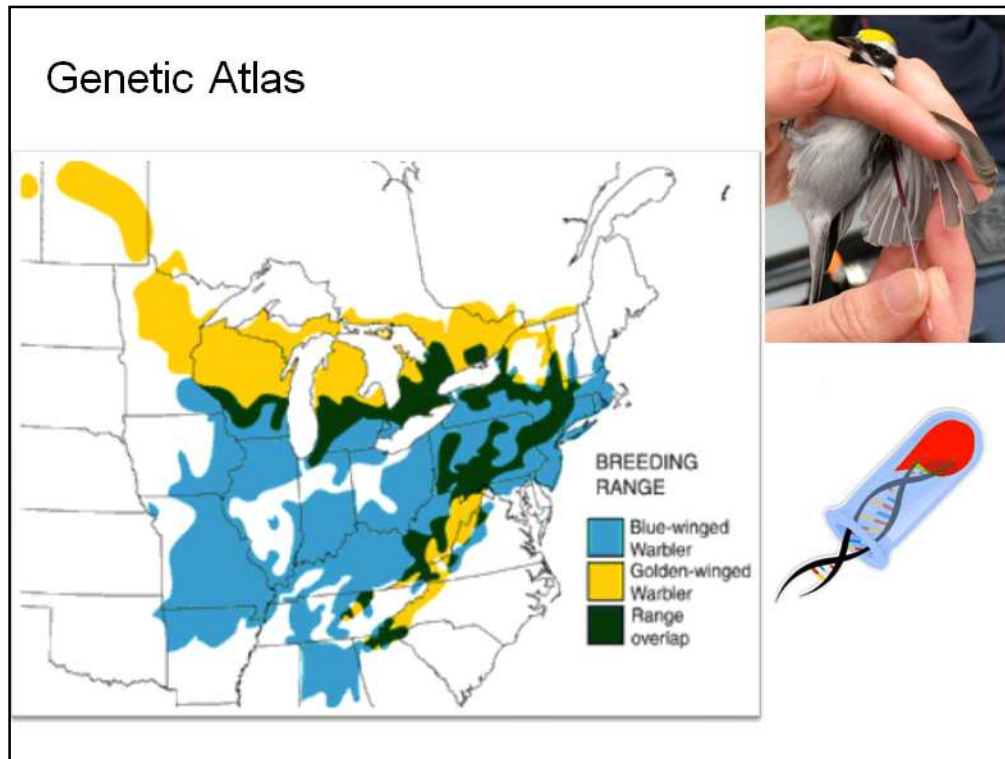
. We use assignment programs to make these determinations as we're using a small sample of genetic markers but we're assuming that it represents the entire genome.



So let's go to that Ontario example I mentioned a few slides ago. Here we have the same pure BWWA and pure GWWA – these can be now used as our reference samples – we can use these as the standards to which we can compare individuals from an area of contact between GWWA and BWWA.

On this other part of the figure we have 76 individuals – all from a breeding population in Ontario and that all look like GWWA. And what we can see is that most individuals are assigned to the ancestral GWWA state, whereas just over 30% are actually assigned to both BWWA and GWWA. All these individuals are considered to be cryptic hybrids.

We were surprised to get these results from this area of active, yet recent hybridization, and it leads us to wonder whether these results are typical of other populations.



The production of a range-wide genetic atlas will be the best tool for the ultimate conservation of this species.

A genetic atlas would use both mtDNA and AFLP data to assess populations throughout the breeding range searching for areas of genetic purity.

Through this process, genetically pure GWWA populations will be identified and targeted for further conservation action, and regions of high introgression will be identified to prevent management that might encourage further expansion by Blue-winged Warblers.

How to generate a genetic atlas:



- STEP 1 - Identify genetically pure populations and use these as your standards

How to generate a genetic atlas:



- STEP 1 - Identify genetically pure populations and use these as your standards
- STEPS 2 & 3 - Isolate informative mitochondrial and nuclear DNA markers

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- STEPS 2 & 3 - Isolate informative mitochondrial and nuclear DNA markers
- STEP 4 - Screen individuals from throughout the breeding range

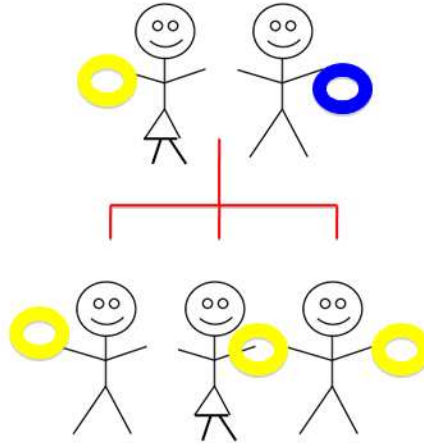
How to generate a genetic atlas:



- STEP 1 - Identify genetically pure populations and use these as your standards
- STEPS 2 & 3 - Isolate informative mitochondrial and nuclear DNA markers
- STEP 4 - Screen individuals from throughout the breeding range
- STEP 5 - Conduct population assignments (STRUCTURE) using your standards as the inferred ancestral populations

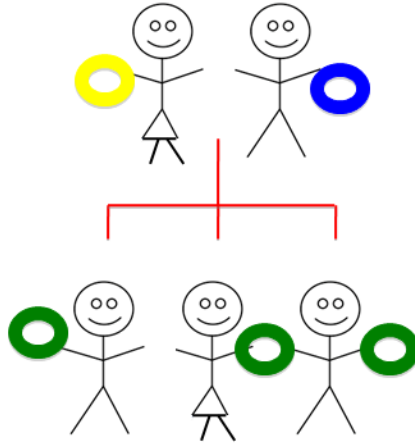
Why 2 types of DNA markers?

- Mitochondrial DNA (mtDNA)
 - maternal inheritance



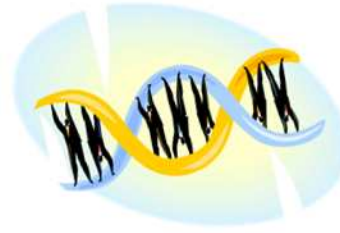
Why 2 types of DNA markers?

- Nuclear DNA (nDNA)
 - biparental inheritance



Why 2 types of DNA markers?

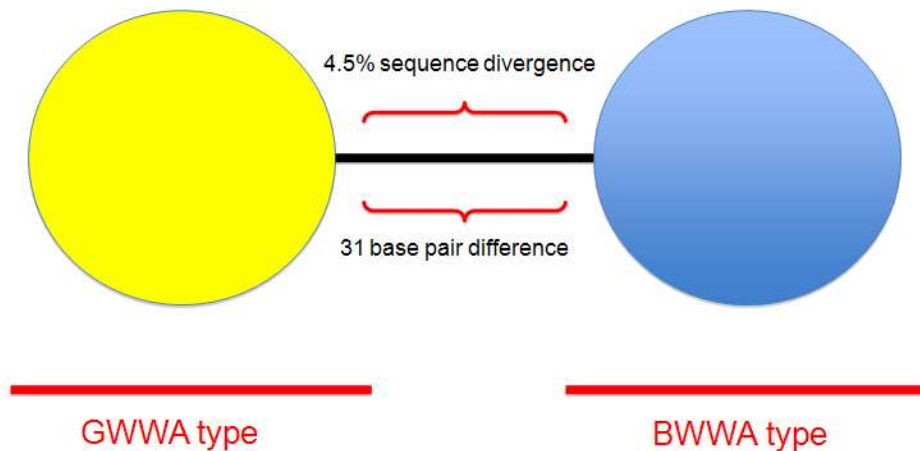
- Enables us to screen multiple genes
- Additional power for analyses
- We can examine both maternal and paternal gene flow



Mitochondrial DNA — markers developed, screening complete.

Nuclear DNA — markers being developed.

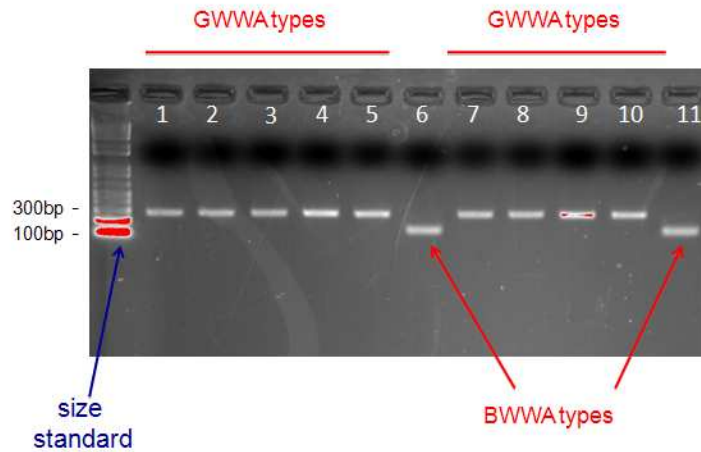
BWWA & GWWA genetic differentiation at NDII (mtDNA locus)



Gill 1997, Shapiro et al 2004, Dabrowski et al 2005, Vallender et al 2007

It was established over 10 years ago that BWWA and GWWA are well differentiated at mtDNA loci. When you screen sections of the mtDNA genome you find that there are types that are distinctly GWWA and types that are distinctly BWWA – represented here by these two circles. In between these types there are 31 base pairs of DNA. This represents about a 4.5% sequence divergence which is on par with the level of differentiation between other wood warbler sister species.

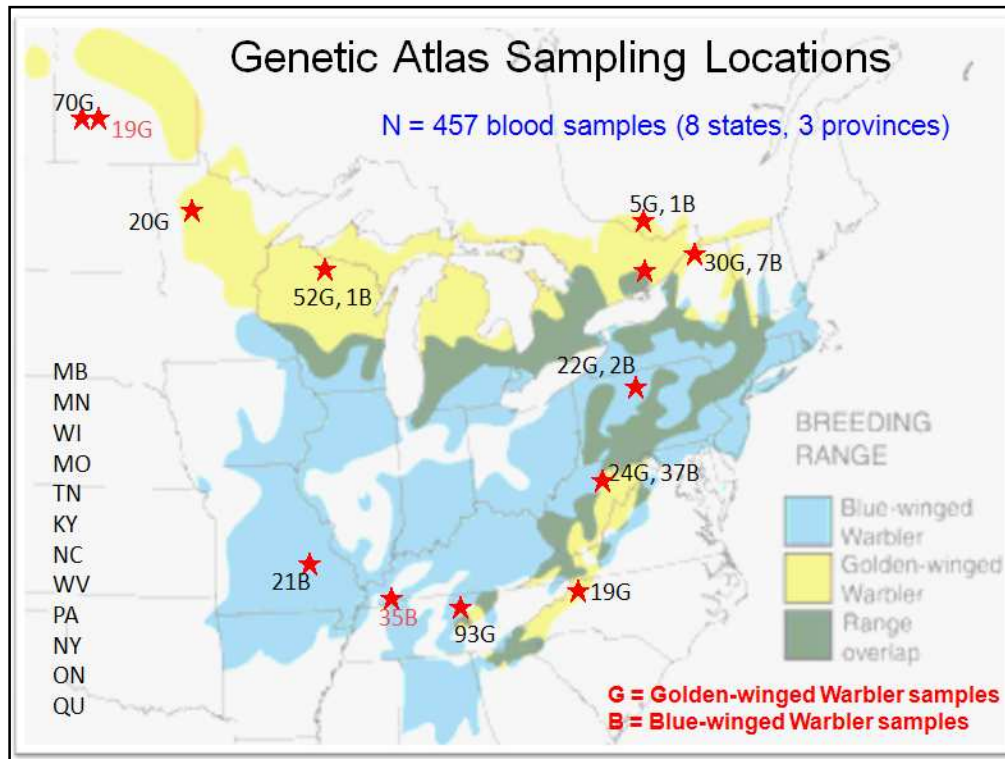
mtDNA screening



Vallender & Lovette, unpub. data

When you screen samples of BWWA and GWWA it's easy to see whether they have either the GWWA type or BWWA type of mtDNA, as shown here on this gel. Here I show 11 individuals – numbers 1 through 5 and then 7 through 10 have GWWA type mtDNA, whereas samples 6 & 11 have BWWA type mtDNA.

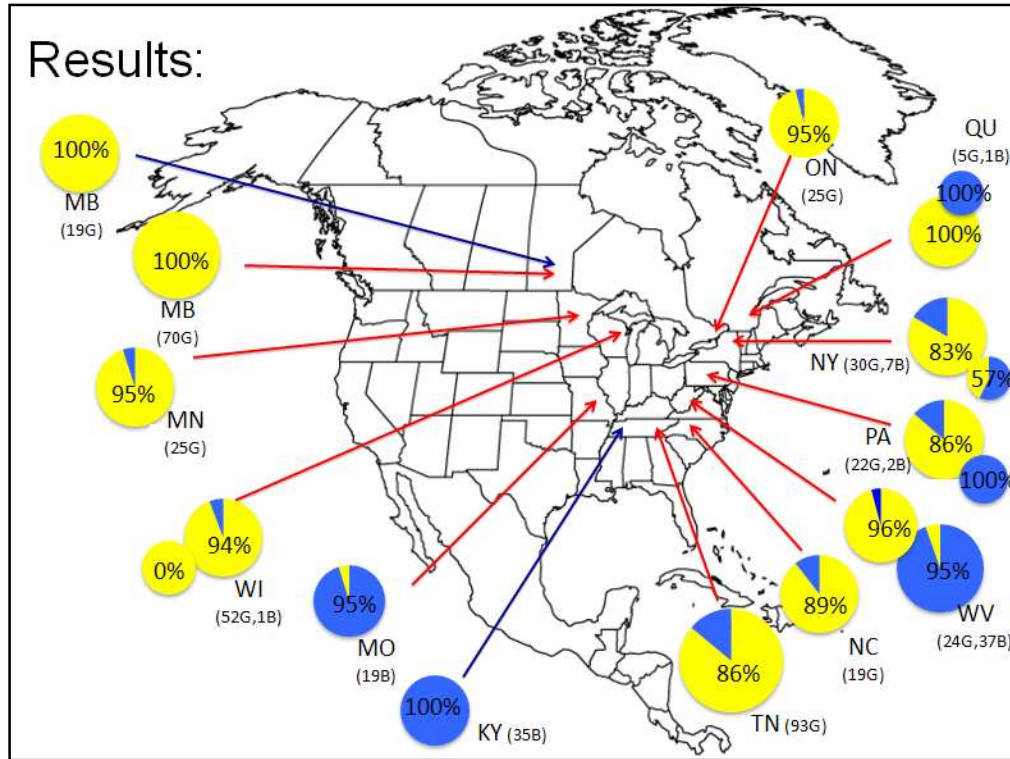
I developed genetic markers from the mtDNA genome that show differences between the species and it's screening with these markers that I've just finished.



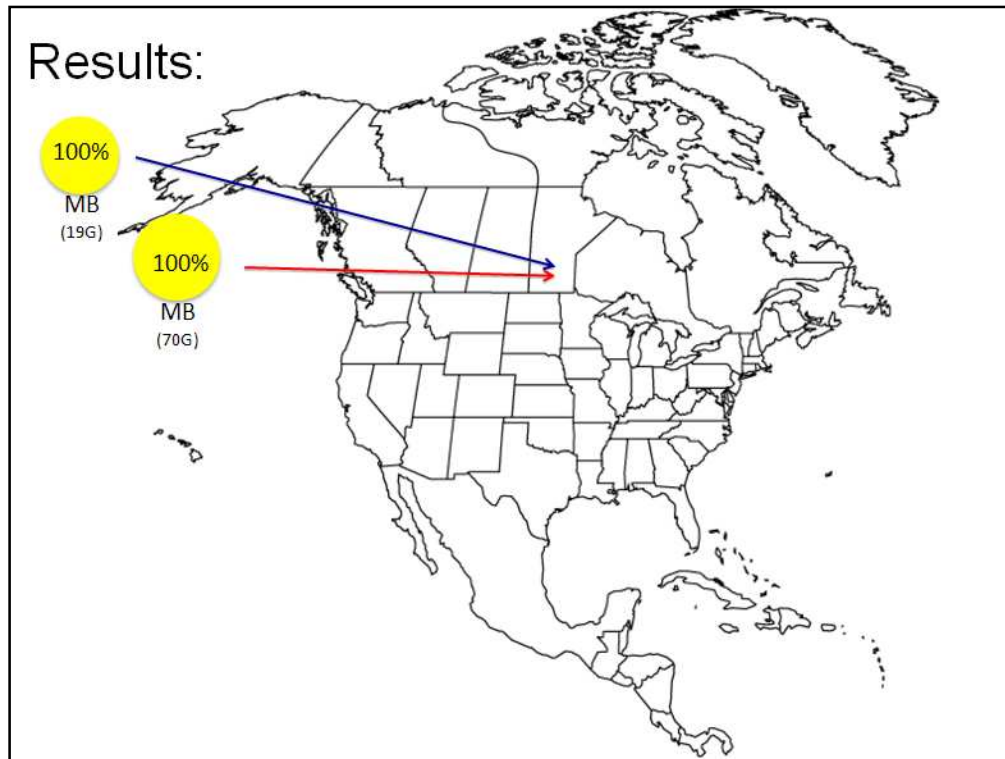
So far I've obtained and screened 457 samples from BWWA and GWWA – from 8 states and 3 provinces.

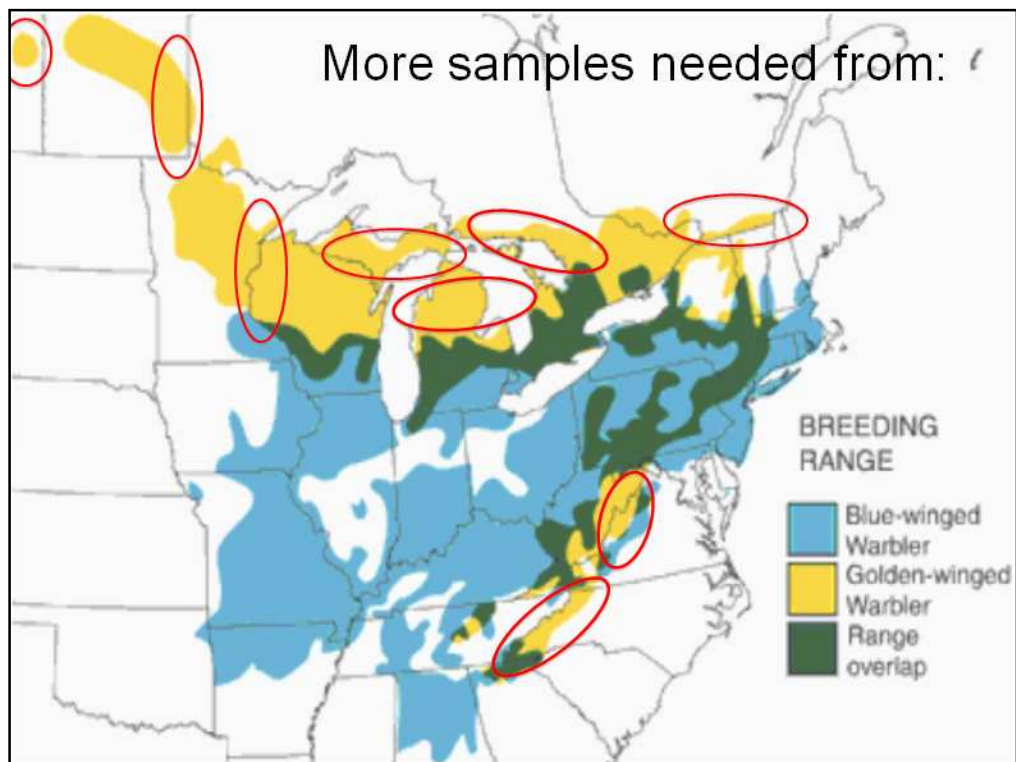
Sample sizes from the various sampling sites are shown here – the numbers followed by G represent GWWA samples and the numbers followed by B represent BWWA samples. The GWWA in MB and KY written in red are the samples I obtained when doing the initial genetic work and that were shown as representing the ancestral populations in previous analyses.

Results:



Results:





How you can help:



- Collect blood or feather samples
- Record phenotype
- Record capture location



Genetic Sampling

We will provide:

1. Blood and feather sampling kit
2. Detailed instruction protocol
3. Potential in-the-field training
4. Protocol to amend bird banding permits (or get a subpermit under Rachel – Canada or the US)

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Blood collectors/suppliers:

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Wildlife Service

Wybo Vanderschuit (MB) – Parks Canada

Jeff Larkin (PA) – Indiana Univ. of PA

Curtis Smalling (NC) – Audubon NC

Ron Canterbury (WV) – U of Cincinnati



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- Joe Chernek, Paul Elsen, Sara Barker Swarthout, Swarthout (NY)
- Nick Luff (MB)



Questions?

